MULTICONJUGATION IN BLEPHARISMA

PAUL B. WEISZ

Arnold Biological Laboratory, Brown University, Providence, R. I.

Conjugation of three or more individuals, i.e. "multiconjugation," has been observed in mass cultures of *Blepharisma undulans.* The phenomenon in this species was first reported by Giese (1938a), and has been seen in a variety of other ciliates during the course of the last century (Chen, 1946). Chen, working on *Paramecium bursaria,* published the first detailed cytological study of multiconjugation. In the present paper an analogous cytological study is described for *B. undulans,* and experiments are set forth indicating how multiconjugation may be brought about.

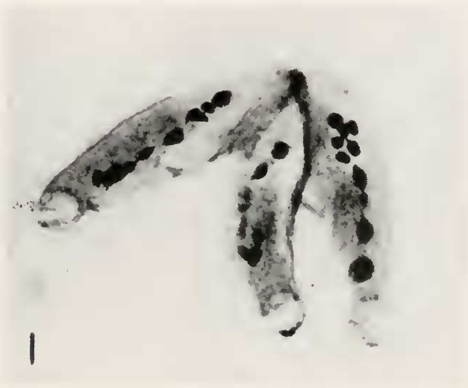
Two stocks of *B. undulans* have been employed, one from the Laboratory at Woods Hole, the other from Elon College, North Carolina. Both of these are morphologically equivalent to Giese's California stock (1938a; also below). The organisms have been cultured at room temperature, with filtered rain water as medium. A rich supply of bacterial food could be maintained in mass cultures by adding a barley seed to each culture dish. For cytological work, given animals have been put on slides, fixed in Carnoy, dried, and stained with methyl green without counterstain (Weisz, 1950).

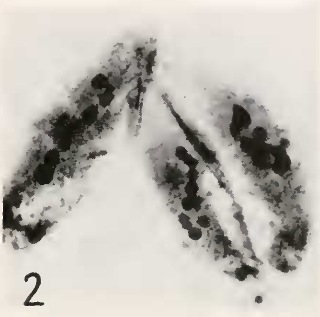
MULTICONJUGATION

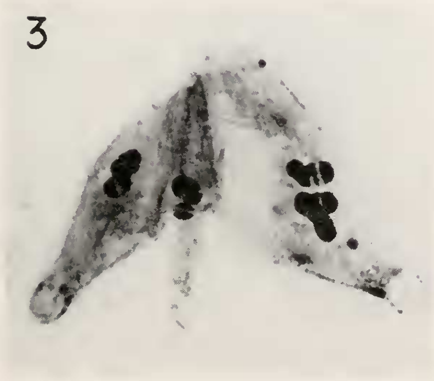
In dense cultures, conjugation epidemics have been observed at intervals, occurring almost simultaneously in different culture dishes and involving perhaps 90 per cent or more of all animals present. Rough counts have shown that approximately 5 per cent to 10 per cent of all matings seen were conjugations of three individuals. Occasionally four, and rarely five, individuals have been found joined into multiconjugant systems. All other conjugations have been paired matings.

Examination of a large number of "triples" has revealed two principal patterns of attachment among conjugants. In one pattern, the three participants are fused together firmly along the full extent of their peristome areas, i.e., from near the anterior tip of the animals almost to mid-level. Attachment is equally extensive between any two of the three members, and the trio suggests a tripod opened up in three dimensions. In another pattern, two of the three conjugants (A and B) are firmly and fully fused as above and as in paired mating, while the third conjugant (C) is peristomially attached to a small area of the peristome of either A or B. In these cases any direct physical connection is not evident between conjugant C and one of the other partners (cf. photographs by Giese, 1938a). Attachment of conjugant C to the posterior body regions of either A or B, as observed by Chen (1946) for *P. bursaria,* has not been seen in *B. undulans.*

In cases of matings involving more than three animals, a large variety of attachment patterns has been noted. Conjugant A might be joined to B, C to A, D to C and/or A, E (if present) to B, or to C, D, or A, etc. Attachment varies in extent but it is always peristomial. It would, therefore, appear generally that conjugative fusion in *B. undulans* always takes place along the peristome area, and that effective fusion need involve only a very small section of this area.







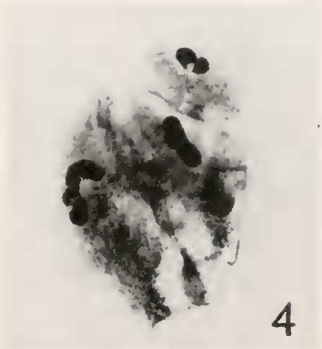


PLATE I

FIGURES 1–4

FIGURE 1. Conjugation of 3 animals, prepared slide, Carnoy-Methyl Green. Animals are joined tripod-fashion. Soon after initial fusion; macronuclear chains beginning to contract. Appr. times 200.

FIGURE 2. Conjugation of 3 animals, prepared slide, Carnoy-Methyl Green. Left individual joined less extensively. Macronuclear contraction under way. Appr. times 200.

FIGURE 3. Conjugation of 3 animals, prepared slide, Carnoy-Methyl Green. Animals joined tripod fashion. After synkaryon formation; in each member two macronuclear primordia visible. Note "kernspalt"-like appearance of primordia. The smaller primordium in each member will degenerate. Appr. times 200.

FIGURE 4. Conjugation of 3 animals, prepared slide, Carnoy-Methyl Green. Animals joined tripod fashion. Late stage of conjugation. In each member only one presumptive macronucleus left; the latter in process of elongation. Appr. times 200.

Multiconjugation typically occurs among animals of equal size, although in many cases one (or more) of the conjugants is smaller than the others. Conjugative fusion lasts as long as in paired mating, i.e., approximately 3 days. After this time the participants separate in groups or singly within a few hours. All the exconjugants are viable and produce vigorous clones.

Cytological

Young (1939) has described in detail the essential cytological steps in normal conjugation of the Yale race of *B. undulans*. The Woods Hole and Carolina stocks differ somewhat from the Yale strain, particularly in the structure and the vegetative behavior of the macronucleus (Weisz, 1949), but the sequence of processes during conjugation is virtually identical to that in the Yale race (Weisz, 1950). The old macronucleus first condenses, fragments, and then degenerates, disappearing completely approximately 36 hours after the conjugants become fused initially. A synkaryon has formed in the meantime, and numerous readily visible, relatively large micronuclei now begin to appear. Simultaneously a number (typically four) of faintly staining macronuclear primordia arise. One of these enlarges, takes up an increasingly dense stain, passes through a "kernspalt"-like stage (Weisz, 1950), and ultimately becomes the definitive macronucleus of a new caryonide. The other primordia degenerate and disappear. During this phase the micronuclei become smaller, and throughout the remainder of the caryonide life history micronuclei are extremely small and often cannot be found at all. It is largely due to this circumstance that synkarya, and pregamic and postzygotic maturation stages cannot be reliably identified. The derivation of micronuclei and of macronuclear primordia from the synkaryon can, however, be inferred by experiment; experimentation has similarly brought out the temporal correlation between micronuclear and the visible macronuclear events (Weisz, 1950). Thus, for any given stage of the macronucleus, the micronuclear condition at that stage can be estimated. It should be noted parenthetically that in typical *B. undulans* only one macronuclear primordium persists, so that a caryonide is defined by the entire clone arising from an exconjugant.

In multiconjugation involving any number of conjugants, characteristic nuclear changes have been consistently observed in every one of the participating animals. This has been found true regardless of the specific pattern of peristomial attachment. The changes are always precisely identical in nature and duration to the cytological sequence observed in paired mating, and they occur synchronously in all members of a multiconjugant system. This is illustrated in Plate I, showing successive stages in the conjugation of three individuals. Since the old macronuclei disappear and new primordia develop, as in paired mating, it may be inferred that synkarya are probably formed in each of the members of a multiconjugant system. In this respect *B. undulans* and *P. bursaria* (Chen, 1946) are similar.

Experimental

In order to determine whether or not multiconjugation is attributable to the existence of multiple mating types, twenty-five single-clone cultures (including both Woods Hole and Carolina stock animals) have been prepared, and individuals from each clone have been mixed with individuals from every other clone. Animals from one of the clones to be tested have first been exposed to light for 1 to 3 days, a procedure which brings out depigmentation of the animals; organisms from another clone were left in the dark, ensuring the retention of the pigmented state (Giese, 1938b). Some 100 animals from each of the two clones have then been transferred together to a deep watch glass containing fresh, relatively food-free culture medium. Under such conditions the members of the two clones can be identified; moreover, transfer to new medium effects a fairly sudden reduction of food organisms, a condition necessary to induce conjugation (Giese, 1938a).

The mixing of clones in Blepharisma, unlike similar procedures in Paramecium, does not result in a striking mating reaction. Nevertheless, after some 20 to 50 hours more than 90 per cent of all animals present are involved in the mating process. This has been found true for mixtures of any two of the twenty-five clones studied. The result confirms similar observations of Giese (1938a). Inspection of each of the mixtures has shown that the majority of conjugation pairs consisted of members from only one of the clones, i.e., in perhaps 60 per cent to 70 per cent of the pairs both conjugants were either deeply pigmented or only lightly pigmented (some restitution of pigment having taken place in the interval). In the remaining mating pairs conjugants of different clones have been involved. In each of the denser mixtures, moreover, a number of multiconjugant systems has been found. Most of these consisted of three animals, usually derived from only one clone. Occasionally, however, four-animal and five-animal conjugations have been seen, and in some of these one or two of the participants derived from the second clone.

In a variation of these tests, organisms from a single clone have been transferred alone to fresh, food-free culture medium. As above, 1 to 2 days after transfer the great majority of animals present have been found in conjugation, and in the denser test cultures a number of multiconjugations could be observed.

Thus it is likely that both the Woods Hole and the Carolina stocks of *B. undulans* are selfing strains; that physiological differentiation into distinct mating types is absent, and that the occurrence of multiconjugation (as of paired conjugation) is primarily a function of food supply and of population density. The age of a clone does not appear to play a decisive role, since conjugation has been induced as early as in the 6th- to 10th-generation descendants of exconjugants.

Discussion

A question of primary interest concerns the basic significance of the observed nuclear changes in multiconjugation. In one of the characteristic patterns in which conjugant A is extensively joined to conjugant B, and C is attached less extensively to B, does conjugant B exchange pronuclei with both A and C? From the manner of attachment it might appear probable that A and B exchange pronuclei as in paired mating, while an autogamous process takes place in conjugant C.

Unfortunately, evidence for the occurrence of autogamy in Blepharisma cannot, at present, be adduced by genetic means (as Sonneborn, 1939, has done for *P. aurelia*), in the absence of mating types, or of other demonstrably gene-controlled characteristics. Moreover, clear-cut cytological evidence for (or against) autogamy is, to date, not available for *B. undulans*, and this is not surprising in view of the inadequacy of the micronuclei for cytological investigation. Macronuclear events are always identical in all conjugative processes, and thus cannot be relied upon to differentiate between autogamy (if it occurs) and true cross-fertilization. Conceivably, an autogamous process need not necessarily be postulated for the second major pattern of multiconjugation, in which A, B, and C are joined in tripod-fashion, the extent of attachment being mutually equivalent among the members. In these cases a pattern of pronuclear exchange might occur, involving all three participants; but the possibility of autogamy is by no means ruled out. In multiconjugations involving four and five individuals, attachment of some of the members is often exceedingly insecure and superficial; these members separate off upon slight agitation very soon after they are initially joined, yet normal conjugative macronuclear changes have been observed in a number of such separated individuals. For these, at least, there probably can be no question of nuclear exchange.

Thus, although direct proof is lacking, the very occurrence of multiconjugation in *B. undulans* probably provides presumptive evidence for the occurrence of autogamy. If so, multiconjugation in this species is cytologically wholly equivalent to this type of "mating" in *P. bursaria* (Chen, 1946).

SUMMARY

A cytological and experimental study was carried out on conjugations involving more than two individuals of *Blepharisma undulans.* Attachment of the participants in a conjugating group occurs in a number of characteristic patterns. Regardless of the attachment pattern, nuclear changes occur in all members of a multiconjugant system. These changes are identical in nature and duration to those observed in normal paired conjugation, and occur synchronously in all participants of the system. The stocks of *B. undulans* used are found to be selfing, physiologically not differentiated into mating types. Multiconjugation can be induced by varying food supply and population density. The probability of autogamous processes in connection with multiconjugation is discussed.

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